



Received on 06 June, 2016; received in revised form, 07 July, 2016; accepted, 09 July, 2016; published 01 December, 2016

OINTMENT OF METHANOLIC EXTRACT OF *FICUS RELIGIOSA*: A TRADITIONAL APPROACH IN WOUND HEALING IN RATS

Reena Gupta* and Jitendra Gupta

Institute of Pharmaceutical Research, GLA University, Chaumuha, Mathura-281406, Uttar Pradesh, India.

Keywords:

Ficus religiosa, Wound healing, Albino rats, Methanolic extract

Correspondence to Author:

Dr. Reena Gupta

Assistant Professor
Faculty Residence Block-10,
Flat No. 404, Institute of Pharmaceutical
Research, GLA University, 17 Km Mile
Stone, NH#2, Mathura-Delhi Road, P.O.
Chaumuha, Pincode-281406, District-
Mathura, Uttar Pradesh, India.

E-mail: rspg80@gmail.com

ABSTRACT: The methanolic extract (ME) of leaves of *Ficus religiosa* (Family: Moraceae) was evaluated for their wound healing activity in rats. It was carried out in rats by employing excision wound models following topical application. The qualitative preliminary phytochemical analysis of ME indicated the presence of tannins, triterpenoids, alkaloids and steroids. All healthy animals of either sex were grouped into four groups and each group contains six rats. The rats of ointment (5 and 10 %w/w) of ME treated were compared to povidone-iodine ointment treated as a standard. The results of present work revealed that the 10%w/w ointment of ME of *Ficus religiosa* treated wounds showed significant reduction of wound contraction area as compared to control and indicated rapid epithelialisation.

INTRODUCTION: Peepal, one of the longest living fast growing deciduous trees has heart shaped leaves shaded in March and April month. Its fruit hidden with the figs and look like the berries that contain the flowers grow in pairs just below the leaves, ripped in the month of May. The juice of leaves employed in an ear troubles, sexual disorders, scabies diarrhea, toothache, cough, migraine, asthma, haematuria and gastric problems; barks in glandular swelling of the neck and inflammations, root bark in clean ulcers, gout, stomatitis and augmented granulations even upon it chew to prevent gum diseases. The ripe fruits employed in heart diseases, thirst and foul taste; and its powered form used to treat asthma. Its seed used in the treatment of urinary troubles¹.

In traditional system of medicine, different parts of peepal like leaves, fruits, vegetative buds, latex, root, aerial roots bark and stem bark have medicinal value and employed in nervous disorders, burns, gynaecological problems, dysentery, diabetes, astringent, vomiting and as a tonic.

The phytochemical investigation of leaves of peepal showed campesterol, tannic acid, isofucosterol, stigmasterol, alanine, threonine, aspartic acid, tryosine, α -amyrin, serine, n-nonacosane, proline, lupeol, isoleucine, arginine, leucine, valine, tryptophan, methionine, glycine, n-octacosan, n-hentricontanen, n-octacosan and hexacosanol²⁻⁸. So the present research work focused on study of wound healing activity of methanolic extract (ME) of *Ficus religiosa*.

MATERIALS AND METHODS:

Collection of Plant Material:

Ficus religiosa plant and leaves were collected in the month of May from local area of Mandsaur and authenticated (Voucher specimen-T/006/2008) by

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.7(12).5006-11
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7(12).5006-11	

professor of botany Dr. C. L. Ningwale, Govt. P.G. College, Mandasaur, M.P., India,

Preparation of Extract:

The fresh shade leaves of plant were collected, dried, ground with the help of grinder and convert into a powder after passing through sieve 80. The fine powder was subjected for extraction in to a soxhlet apparatus by using methanol at room temperature. The mixture was filtered with the help of whatman filter paper and kept the filtrate for drying at 40°C in an oven then calculate the percent yield of extract. The methanolic extract was stored in air tight containers for further study⁹⁻¹⁰.

Preliminary Phytochemical Study:

The presence of alkaloids, tannins, steroids, triterpenoids, glycosides and flavanoids active phytochemical constituents was accessed in methanolic extract (ME) of *Ficus religiosa*⁹⁻¹⁷.

Ointment Preparation:

Ointments of ME of *Ficus religiosa* (5 and 10 % w/w) were developed after incorporation of methanolic extract in to the ointment base IP¹⁸.

Pharmacological Wound Healing Activity Study:

Experimental Animals:¹⁹⁻²⁰

The *in-vivo* wound healing activity was carried out in wistar rats of either sex weighing between 120-200g by using excision wound model. The protocol of the present work was approved by Institutional Animal Ethical Committee (IAEC), Kota College of Pharmacy, Kota, Rajasthan, India (Ref.No.KCP/1291/09/ac/CPCSEA). The procure animals were grouped and housed in poly acrylic cages (38x23x10 cm) with not more than four animals per cage and maintained under standard laboratory conditions with natural dark and light cycle (14 hrs light/10 hrs dark) at 27±2°C and relative humidity (RH) 44-56%. They were allowed free access to standard diet (Golden Feeds, India) and tap water *ad libitum* for one week before and during the experiments. The animals were acclimatized one week before start the activity in laboratory.

***In-vivo* Excision Wound Model:** *In-vivo* wound healing activity was carried out by using excision

wound model to investigate percent wound contraction (PWC) and epithelization²¹⁻²⁴. The animals were anaesthetized with ketamine hydrochloride (1ml, 10mg/kg I.V.), depilated on back and inflicted with an excision of 500 mm² as explained in Morton and Malone²⁵. Blotting the wound by swabbing with cotton and achieved the homeostasis.

The randomly selected animals were grouped into four groups each group have six albino rats Wistar strain of 6-8 weeks of age of either sex, each weighing 120-200g. The following treatments were received by the rats and once in a day covered the entire area of wound till complete healing occurred as mention below:

Group I - Served as control group and received no treatment.

Group II- Served as standard group and received application of povidone-iodine ointment (5%w/w).

Group III- Served as treated group and received application of ointment (5%w/w) of ME of *Ficus religiosa* (test formulation).

Group IV- Served as treated group and received application of ointment (10%w/w) of ME of *Ficus religiosa* (test formulation).

The respective treatments were received by the animals from starting zero '0' day till the completion of wound healing take place. PWC was determined at different time interval 6, 12 and 18 days. Trace areas of wounds were measured planimetrycally with the help of graph paper (mm scale). Without any residual raw wound, number of days needed for falling of the scab provided the period of epithelization. PWC was calculated with the help of following equation-

$$PWC = \{A_H/A_{TW}\} \times 100$$

A_{TW}-Total wound area, A_H -Healed area, PWC-Percent wound contraction,

Statistical analysis:

The results of pharmacological studies were expressed as Mean ± S.D. The total variations present in data were evaluated by using Graph Pad

Prism 5 project software ANOVA (analysis of variance) followed by Student's t-Test. The result were considered statistically significant when p value less than 0.05 ($P < 0.05$) vs control.

RESULT AND DISCUSSION:

After extraction of leaves of *Ficus religiosa*, investigated the qualitative preliminary phytochemical analysis of ME indicated the presence of tannins, triterpenoids, alkaloids, steroids, etc and absent of flavonoids and steroids, result showed in **Table 1**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL STUDY OF ME (METHANOLIC EXTRACT) OF *FICUS RELIGIOSA*.

Test	Reagents	Observation	Inference
Glycosides			
a. Borntrager's test	ME+ Conc. H ₂ SO ₄ +Benzene or CHCl ₃ +Ammonia	Ammonical layer become red or pink	+
b. Modified Borntrager's test	ME+5% FeCl ₃ (5ml) + Dil.HCl (5ml)+Benzene+Dil. Ammonia	Ammonical layer become pinkish red	+
c. Barfoed's test	ME+Barfoed reagents	Red colour (monosaccharide) after 10 min. colour form (disaccharide)	+
Alkaloids			
a. Mayer's test	ME+Potassium mercuric iodide solution	Cream precipitate	+
b. Dragendroff's test	ME+Potassium bismuth iodide solution	Reddish brown precipitate	+
c. Wagner's test	ME+Iodine potassium solution	Brown precipitate	+
d. Hager's test	ME+Saturated solution of picric acid	Yellow colour	+
Flavonoids			
a. Shinoda Test	ME+95% ethanol(5ml) + drops of conc. HCl+ 0.5g magnesium	No Change	-
b. Ferric chloride test	ME+ few drops of neutral FeCl ₃	No Change	-
Steroids			
a. Salkowski test	ME+Chloroform+Conc. H ₂ SO ₄	No Change	-
b. Liebermann Burchard test	ME+Chloroform+Acetic anhydride +Conc. H ₂ SO ₄	No Change	-
Tannin			
a. Lead acetate Test	ME+ 5ml, distilled water + few drops of (1%) Lead acetate solution	White precipitate	+
b. Ferric chloride test	ME+FeCl ₃ (1%)	Blue, green or brownish green colour	+
Terpenoids			
a. Salkowski Test	ME+Chloroform+Conc. H ₂ SO ₄	Yellow colour	+
b. Noller's Test	ME+Tin+ 1 ml Thionylchloride	Purple colour	+

ME- Methanolic extract, (+)- Presence, (-)- Absent

In the present *in-vivo* study, after the topical application of ointment of ME of *Ficus religiosa* the excision wound model was employed for the investigation of wound contraction and epithelization period. When compare the test ointment formulation with iodine-povidone ointment as a standard and normal healing as a control, a significant reduction in the wound contraction area was found from 6,12 and 18 post wound days. This study was performed until the fall of scar leaving on raw material behind, result of wound healing activity showed in **Table 2** and **Fig. 1-2**. So from excision wound study, it was investigated that initially from day '0' onward no

too much difference was observed in wound contraction (WC) in these groups I, II, III and IV. But as well as time passage after 9 day WC was rapid in treated group III (81.77±2.09) and group IV (96.15±1.44) at 18 days when results were compared with Group I (69.49±3.88) at 18 days and lesser than Group II (98.34±2.51) at 18 day.

We observed that comparative potential of both standard and tests (5% w/w, 10% w/w) formulations showed complete healing on 20.19±0.47, 24.74±0.56 & 21.53±0.31 days respectively and 30.38±0.56 days for untreated group.

TABLE 2: EFFECT OF OINTMENT OF ME ON EXCISION WOUND MODEL IN RATS.

Group	Treatment	Time Interval (Days)	PWC (%) [#]	Time of Epithelization (Days) [#]
Group I	Control	6	4.27±2.31	30.38±0.56
		12	18.15±1.98	
		18	69.49±3.88	
Group II	Standard 5% w/w	6	29.32±2.63**	20.19±0.47***
		12	61.53±2.35***	
		18	98.34±2.51***	
Group III	Test 5% w/w	6	21.47±1.42**	24.74±0.56***
		12	39.75±2.14***	
		18	81.77±2.09***	
Group IV	Test 10% w/w	6	27.10±1.11**	21.53±0.31***
		12	57.23±3.10***	
		18	96.15±1.44***	

Values are represented as Mean±SEM, [#]N=6. * P<0.05, **P<0.01 and ***P<0.001 compared to control. PWC- Percent Wound Contraction

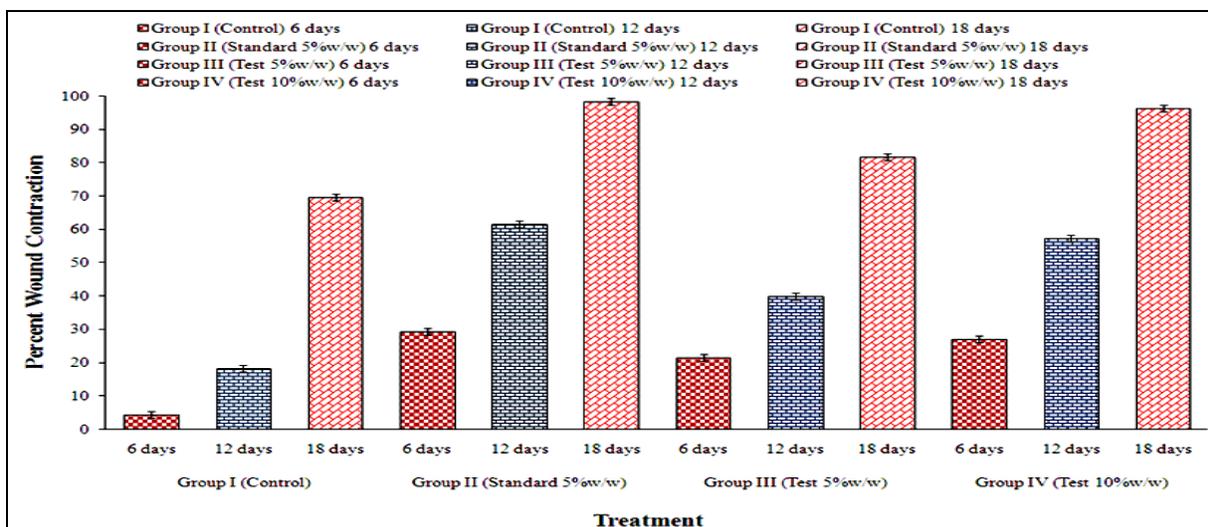


FIG. 1: EFFECT OF STANDARD AND OINTMENT (5AND 10%W/W) OF METHANOLIC EXTRACT ON EXCISION WOUND MODEL IN RATS.

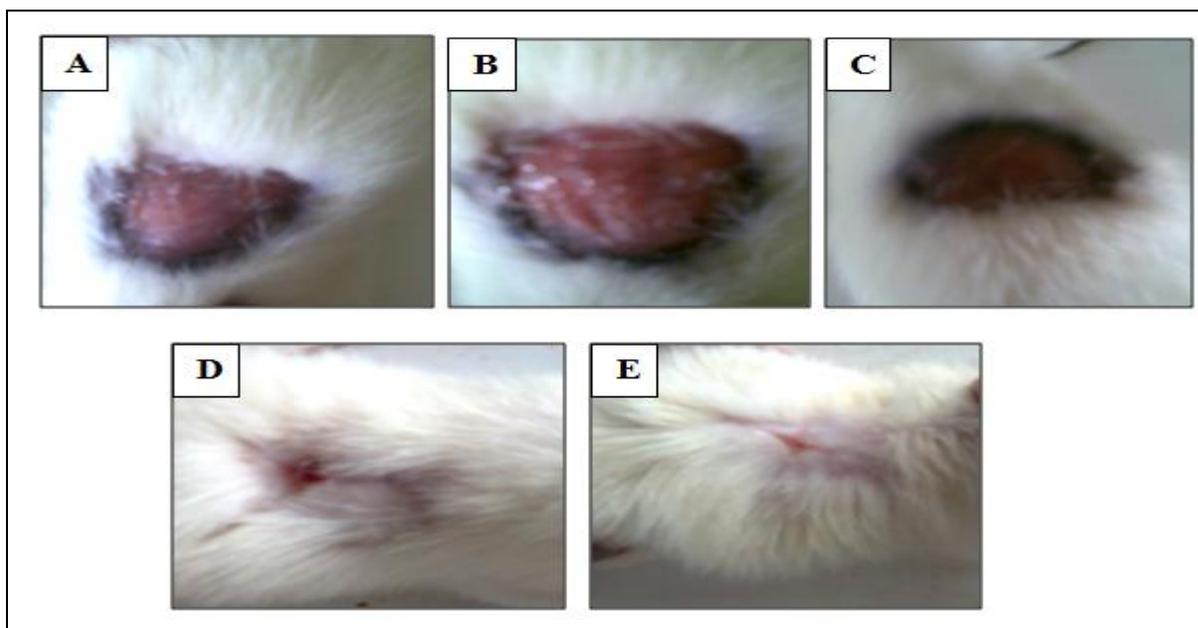


FIG. 2: PHOTOGRAPHIC REPRESENTATION OF WOUND HEALING, A-DAY 1 (CONTROL), B-DAY 1 (TEST 10%W/W), C-DAY 1 (STANDARD), D- DAY 18 (TEST 10%W/W) AND E- DAY 18 (STANDARD).

CONCLUSION: In conclusion, the phytochemical analysis clearly stated that leaves contained various active phytoconstituents like glycoside, alkaloid, tannin and steroids and absence of flavanoids and steroids. Wound healing comprise a highly dynamic concatenated order of cellular physiological & biochemical phenomenon that do not required special care or help but wounds are prone to infection and cause discomfort. So wound healing agent is needed to heal up the wound. Wound healing become delay or incomplete in case of ischemia, diabetes and burn due to tissue necrosis etc or in severe conditions. Therefore in such special situation wound healing agent is needed that promote the healing.

The concurrent and independent various biological process granulation, collagenation, collagen maturation and scar maturation play important role in wound healing process. Finally concluded that test treated group significantly enhances the wound contraction rate with respect to standard, control group and epithelization. The collagens not only related to strength but also related to tissue integrity and maintain the homeostasis and later on epithelization of healing of wound. So in rats proliferation of collagen by employing of ME may contribute significantly to healing of wound may be due to tannins, other phytoconstituents and also impart necessary strength to repair the tissue^{26, 27}. So in the present research, excision wound model was employed to access the traditionally effect of ointment of ME of leaves of *Ficus religiosa* on wound healing²¹⁻²⁴.

ACKNOWLEDGEMENT: Authors are thankful to Dr. Govind Mohan, Retired professor, S.N. Medical college, Agra and Dr. C. L. Ningwale, Govt. P.G. College, Mandsaur, M.P., India, for help.

REFERENCES:

1. Shailja S and Jaiswal S: Therapeutic properties of *Ficus religiosa*. International Journal of Engineering Research and General Science 2014; 2(5):149-158.
2. Joseph B and Justin SR: Phytopharmacological and phytochemical properties of three *Ficus species*-an overview. International Journal of Pharma and Bio Sciences 2010; 1(4): 246-253.
3. Margareth BCG and Miranda JS: Biological activity of Lupeol. International journal of Biomedical and Pharmaceutical Sciences 2009; 3(1): 46-66.
4. Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z and Singh AK: Dictionary of Indian Medicinal Plants. CIMAP Lucknow, India, 1992: 546.
5. Makhija IK, Sharma IP and Khamar D: Phytochemistry and pharmacological properties of *Ficus religiosa*: an overview. Annals of Biological Research 2010; 1(4): 171-180.
6. Panda SK, Panda NC and Sahue BK: Phytochemistry and pharmacological properties of *Ficus religiosa*: an overview. Indian Veterinary Journal 1976; 60:660-664.
7. Verma RS and Bhatia KS: Chromatographic study of amino acids of the leaf protein concentrates of *Ficus religiosa* Linn. and *Mimusops elengi* Linn. Indian Journal of Hospital Pharmacy 1986; 23:231-232.
8. Behari M, Rani K, Usha MT and Shimiazu N: Isolation of active-principles from the leaves of *Ficus religiosa*. Current Agricultural 1984; 8:73.
9. Gupta R, Gupta MK, Bhandari A and Gupta J: Preliminary pharmacognostical and physicochemical analysis: a poly herbomineral formulation. International Journal of Drug Development and Research 2014; 6(3):85-92.
10. Gupta R, Gupta MK, Bhandari A, Gupta J and Pathan IK: Preparation and standardization of polyherbomineral formulation. International Journal of Drug Development and Research 2014; 6(2):211-219.
11. The Ayurvedic Pharmacopoeia of India. Ministry of Health and Family Welfare, Government of India, New Delhi, Part-I, Volume-III, 2001: 29-30,133.
12. Standardization of Single drugs of Unani Medicine. Published by Central Council For Research in Unani Medicine, Ministry of Health and Family Welfare, Government of India, New Delhi, Part III, 1987: 24,85-88,147-151,152-157,189.
13. Khandelwal KR: Practical Pharmacognosy: Techniques and experiments. Nirali Prakashan, Pune, India, Edition 14, 2005: 149-155.
14. Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy. Nirali Prakashan, Pune, India, Edition 12, 1999: 181,213, 216, 224, 279, 315, 322, 390, 425-427, 593-597.
15. Harborne J B: Phytochemical methods. Jackman H. (Ed.), London, 1973: 70.
16. Gupta R, Gupta RD, Chatap VK, Sharma DK and Gupta VB: Tapioca starch as a directly compressible tablet excipient. International Journal of Green Pharmacy 2006; 1(1):62-63.
17. Gupta R, Jain AK, Sagar R, Jain A, Jain A and Sikarwar MS: Ayurveda and liver care. Sachitra Ayurveda 2006: 531-534.
18. Odimegwu DC, Ibezim EC, Esimone CO, Nworu CS and Okoye FBC: Wound healing and antibacterial activities of the extract of *Dissotis theifolia* (Melastomataceae) stem formulated in a simple ointment base. Journal of Medicinal Plants Research 2008; 2(1):011-016.
19. Gupta J, Mohan G, Prabakaran L and Gupta R: ACF loaded ethyl cellulose microspheres: formulation designing, characterization and *in-vivo* antiinflammatory and analgesic activities in albino wistar rats. International Journal of Drug Development and Research 2014; 6(3):133-144.
20. Gupta R, Gupta MK, Bhandari A and Gupta J: Evaluation of antitussive activity of polyherbomineral formulation on cough reflex induced by different cough induced models in mice. International Journal of Drug Development and Research 2014; 6(4):280-289.
21. Kumari P, Chahar MK, Veerapur VP, Spandana G, Thippeswamy BS and Badami S: Spider web ointment: a

- traditional base approach in cutaneous wound healing. Indian Journal Traditional Knowledge 2013; 12(4):657-663.
22. Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK and Dhawan BN: *In-vitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica*. Journal of Ethnopharmacology 1999; 65:1-11.
 23. Ingold WM: Wound therapy: growth factors as agent to promote healing. Trends in Biotechnology 1993; 11:387-392.
 24. Silambujanaki P, Chandra CHBT, Kumar KA and Chitra V: Wound healing activity of *Glycosmis arborea* leaf extract in rats. Journal of Ethnopharmacology 2011; 134:198-201.
 25. Morton JJP and Malone MH: Evaluation of vulnerary activity by an open wound procedure in rats. Archives Internationales De Pharmacodynamie Et De Thérapie 1997; 196:117-26.
 26. Charde RM, Dhongade HJ, Charde MS and Kasture AV: Evaluation of antioxidant, wound healing and anti-inflammatory activity of ethanolic extract of leaves of *Ficus religiosa*. International Journal of Pharmaceutical Science and Research 2010; 1:73- 82.
 27. Roy K, Shivakumar H and Sarkar S: Wound healing potential of leaf extracts of *Ficus religiosa* on wistar albino strain rats. International Journal of PharmTech and Research 2009; 1:506-508.

How to cite this article:

Gupta R and Gupta J: Ointment of Methanolic Extract of *Ficus Religiosa*: A Traditional Approach in Wound Healing in Rats. Int J Pharm Sci Res 2016; 7(12): 5006-11. doi: 10.13040/IJPSR.0975-8232.7(12).5006-11.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)